View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

New and Notable

Shedding Light on Conformational Dynamics of Na^+-Coupled Transporters

Christof Grewer*
Binghamton University, Department of Chemistry, Binghamton, New York

Na^+-driven solute carriers (SLCs) for physiologically important organic and inorganic substrates are thought to operate on the principle of alternating access. The alternating access mechanism predicts that the substrate binding site of the transporter can be exposed to either the extracellular or the intracellular side of the membrane, but not to both sides at the same time (1). Direct evidence for this mechanism has been obtained recently from three-dimensional structures of several families of active transporters, which were crystallized in varying conformations, including inward- and outward-facing states (reviewed in Forrest et al. (2)).

Na^+-driven transporters for inorganic phosphate are known to belong to the SLC20 and SLC34 families (3). The SLC34 family member NaPi-IIa is expressed in the apical membranes of the proximal tubule in the kidney, where it contributes to phosphate reuptake (3). NaPi-IIa utilizes the transmembrane concentration gradient of Na^+ for concentrative uptake of phosphate against its own concentration gradient. The structure of the phosphate transporters is currently not known. However, analysis of the transmembrane topology of NaPi-IIa indicates the existence of an inverted repeat (3), a hallmark of the molecular architecture of several secondary-active transporters (for example, see Abramson et al. (4)).

Transport via the alternating access mechanism requires that transporters visit distinct conformational states while moving through the transport cycle. Direct determination of the structures of these states is desirable. However, obtaining such structures remains a formidable challenge. In addition, crystal structures represent only a static image. However, information on the dynamics of transitions between distinct states, as well as the steady-state distribution of states, is critical for a detailed understanding of the transport mechanism.

In this edition of the *Biophysical Journal*, Patti and Forster (5) present elegant work that combines electrophysiological analysis of NaPi-IIa transporter function with fluorescence measurements from the same cell to characterize conformational changes in the transport cycle (5). Using this voltage-clamp fluorometry (VCF) approach, the steady-state distribution of conformational states was perturbed by step changes in the transmembrane potential. Subsequently, relaxation to a new steady state was followed by recording emission from a fluorescent reporter that was sensitive to the microenvironment, and that was attached to NaPi-IIa at strategically-selected positions. Interestingly, voltage-jump-induced fluorescence changes were dependent on the exact location of labeling, with two sites showing opposing signs of fluorescence changes at the same voltage. These results suggest that voltage-dependent conformational changes lead to specific changes in the microenvironment at these labeled sites.

VCF has been used in the past for functional studies of channels and transporters (6,7). What makes the report by Patti and Forster unique is the meticulous analysis of the fluorescence and electrophysiological data, using kinetic simulations. The authors arrive at a unified mechanistic model that fully accounts for the experimental data, at the same time integrating previous results from mechanistic studies. They propose that conformational transitions of the phosphate and Na^+-free transporter, as well as Na^+ binding to the internal and external binding sites, are associated with charge redistribution within the membrane. The reporter fluorophores were attached to positions at the top of predicted transmembrane domains 1 and 2, although other positions at the extracellular boundaries of transmembrane domains 3, 6, and 10 also report on voltage-dependent structural changes, as published by the same laboratory previously (8). Finally, the results allowed the authors to predict fluorescence intensities of the attached fluorophores at different positions and in different states along the transport cycle, yielding state distributions consistent with the alternating access hypothesis. These state distributions are controlled by the transmembrane potential and the concentration of the driving Na^+ ion.

The VCF technique, as clearly demonstrated in this report, has the potential to provide detailed information on the conformational dynamics of transport proteins, which are unique to the position of attachment of the fluorophore. The structure of NaPi-IIa is unknown, making the structural interpretation not straightforward. However, for transporters, for which the three-dimensional structure is known, this approach should yield mechanistic information that is even more detailed. Thus, it is likely that VCF will continue to be a powerful tool in research on transporter mechanisms.

REFERENCES

1. Jardetzky, O. 1966. Simple allosteric model for membrane pumps. *Nature.* 211:969–970.
2. Forrest, L. R., R. Krämer, and C. Ziegler. 2011. The structural basis of secondary active transport mechanisms. *Biochim. Biophys. Acta.* 1807:167–188.
3. Biber, J., N. Hernando, and I. Forster. 2013. Phosphate transporters and their function. *Annu. Rev. Physiol.* 75:535–550.

Submitted January 29, 2014, and accepted for publication February 25, 2014.

*Correspondence: cgrewer@binghamton.edu

Editor: Ernest Wright.

© 2014 by the Biophysical Society

0006-3495/14/04/1549/2 $2.00

CrossMark

http://dx.doi.org/10.1016/j.bpj.2014.02.029

4. Abramson, J., I. Smirnova, …, S. Iwata. 2003. Structure and mechanism of the lactose permease of *Escherichia coli*. *Science.* 301:610–615.

5. Patti, M., and I. C. Forster. 2014. Correlating charge movements with local conformational changes of a Na^+-coupled cotransporter. *Biophys. J.* 106:1618–1629.

6. Meinild, A. K., B. A. Hirayama, …, D. D. Loo. 2002. Fluorescence studies of ligand-induced conformational changes of the Na^+/glucose cotransporter. *Biochemistry.* 41: 1250–1258.

7. Li, M., R. A. Farley, and H. A. Lester. 2000. An intermediate state of the γ-aminobutyric acid transporter GAT1 revealed by simultaneous voltage clamp and fluorescence. *J. Gen. Physiol.* 115: 491–508.

8. Virkki, L. V., H. Murer, and I. C. Forster. 2006. Mapping conformational changes of a type IIb Na^+/Pi cotransporter by voltage clamp fluorometry. *J. Biol. Chem.* 281: 28837–28849.